

We claim:

1. A method for determining if a test compound induces uracil UTP into DNA, the method comprising:

- 5 a) providing aliquots of the following cells:
- i) wildtype cells;
 - ii) cells overexpressing a duties;
 - iii) cells overexpressing a UT1 glycosylase; and
 - iv) cells expressing the UT1 glycosylase inhibitor protein Ugi or cells
- 10 possessing a compromised UT1 glycosylase function;
- b) exposing the cells to an agent that directly or indirectly inhibits thymidylate metabolism, in the presence or absence of the test compound;
- c) measuring one or more features of the exposed cells, the features comprising:
- 15 i) cell growth or viability;
 - ii) cell cycle checkpoint arrest;
 - iii) presence of replication intermediates in the cells;
 - iv) amount of DTP present in the cells; and
 - v) presence or amount of uracil in DNA of the cells; and
- d) interpreting the measured features, wherein a profile in the four cell types which
- 20 is indicative that the test compound induces uracil mis-incorporation into DNA comprises one or more features in each of the cell types comprising:
- i) in the wildtype cells, cytotoxicity, cell cycle arrest at G1/S or early S phase, presence of replication intermediates, elevated DTP pools or little or no detectable uracil in the DNA;
 - 25 ii) in the duties overexpressing cells, enhanced resistance to cytotoxicity, cell cycle arrest at mid S-phase, presence of replication intermediates, low DTP pools, or little to no detectable uracil in DNA
 - iii) in the UT1 glycosylase overexpressing cells, cytotoxicity or enhanced

cytotoxicity, cell cycle arrest at G1/S or early S-phase, presence of replication intermediates, elevated DTP pools, or little to no detectable uracil in DNA; and

iv) in the nonfunctional UT1 glycosylase cells, enhanced resistance to cytotoxicity, cell cycle arrest at G2/M phase, reduced presence of replication intermediates, elevated DTP pools, or stable uracil incorporation into DNA.

2. The method of claim 1, wherein the cells are of an organism selected from the group consisting of yeast, *D. melanogaster*, and *C. elegans*.

3. The method of claim 2, wherein the cells are yeast cells and the conversion of dUMP to TMP is inhibited by an antifolate.

4. The method of claim 3, wherein the antifolate is selected from the group consisting of aminopterin and sulfanilimide.

5. The method of claim 1, wherein the cells overexpress a duties from an organism selected from the group consisting of humans, animals, plants, fungi, algae, protozoa, bacteria and viruses.

6. The method of claim 1, wherein the cells overexpress a UT1 glycosylase from an organism selected from the group consisting of humans, animals, plants, fungi, algae, protozoa, bacteria and viruses.

7. The method of claim 1, wherein the cells lacking a UT1 glycosylase function are produced by producing in the cells an inhibitor of UT1 glycosylase.

8. The method of claim 1, wherein the inhibitor of UT1 glycosylase is obtained from a virus.

9. The method of claim 1, adapted for determining if the test compound inhibits duties, the adaptation comprising, in step (d), observing in each of the four cell types one or more features comprising:

5 i) in the wildtype cells, cytotoxicity, cell cycle arrest at G1/S or early S phase, presence of replication intermediates, elevated DTP pools, or little or no detectable uracil in the DNA;

10 ii) in the duties overexpressing cells, continued growth resistance to cytotoxicity, cell cycle arrest not present or, if present, occurring at mid S-phase, presence or absence of replication intermediates, low DTP pools, or little to no detectable uracil in DNA;

15 iii) in the UT1 glycosylase overexpressing cells, cytotoxicity or enhanced cytotoxicity, cell cycle arrest at G1/S or early S-phase, presence of replication intermediates, elevated DTP pools, or little to no detectable uracil in DNA; and

20 iv) in the UT1 glycosylase inhibitor (Ugi) expressing cells, enhanced resistance to cytotoxicity, cell cycle arrest at G2/M phase, reduced presence of replication intermediates, elevated DTP pools, or stable uracil incorporation into DNA.

10. The method of claim 1, wherein two or more test compounds are added to the aliquots of cells.

11. A kit comprising:

a) aliquots of the following cells:

i) wildtype cells;

25 ii) cells overexpressing a duties;

iii) cells overexpressing a UT1 glycosylase; and

iv) cells lacking a UT1 glycosylase function; and

b) instructions for using the cells in an assay to determine if a test compound

induces uracil UTP into DNA.

12. A method for determining effectiveness in a patient of chemotherapy targeting conversion of dUMP to TMP, the method comprising:

- 5 a) obtaining from the patient a sample of cells which are the target of the chemotherapy;
- b) measuring one or more features of the cells, the features comprising:
- i) cell growth or viability;
- ii) cell cycle checkpoint arrest;
- 10 iii) presence of replication intermediates in the cells;
- iv) amount of DTP present in the cells; and v) presence or amount of uracil in DNA of the cells; and
- c) observing if one or more of the measured features is the same as or differs from features comprising: cytotoxicity, cell cycle arrest at G1/S or early S-phase, presence of
- 15 replication intermediates, elevated DTP pools or little or no detectable uracil in the DNA, wherein a lack of divergence from one or more of the features is indicative that the chemotherapy is effective, and a divergence from one or more of the features indicates a possibility that the chemotherapy is of reduced effectiveness or is ineffective.